Report

Kinetic Study of Organic Reactions on Polystyrene Grafted MicroTubes

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Kinetic Study of Organic Reactions on Polystyrene Grafted MicroTubes

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Solid-phase organic synthesis (SPOS)[1] has been used broadly to assemble small molecule combinatorial libraries for drug discovery.[2] Commonly, solid-phase organic reactions are performed on either cross-linked polystyrene/TentaGel resin beads or on polystyrene (PS) grafted polypropylene MicroTubes/Crowns. Recently, both types of solid supports have been applied in library syntheses using radio frequency (RF) tagged MicroKan or MicroTube reactors.[3,4]

As a novel type of solid support, polystyrene grafted polypropylene MicroTubes have been modified with a variety of functional groups and linkers such as chloromethyl (Merrifield type), 4-hydroxymethylphenoxy (Wang type), aminomethyl (AM type), and Knorr linker. A combinatorial library of tyrphostins has been described using aminomethylated MicroTubes.[4] Recently, chloromethyl MicroTubes were introduced and applied in solid-phase organic syntheses.[5] In addition, polystyrene grafted fluoropolymer MicroTubes were reported and used in high-temperature applications.[6]

The physical and chemical properties of grafted polymeric surfaces such as MicroTubes and Crowns differ from those of cross-linked resin. The reaction kinetics of polystyrene and TentaGel based resin beads have been compared, and the results contradict the popular presumption that solid-phase reactions perform more readily on “solution-like” TentaGel resins.[7−9] Recently, various cross-linked polystyrene resins were compared using the attachment of Knorr linker as a model reaction.[10] In this paper, we report the kinetic studies of six common organic reactions on polystyrene grafted MicroTubes.

Results and Discussion

I. Organic Reactions on Wang MicroTubes. 1. Oxidation with IBX and Reduction with LiBH$_4$. As shown in Scheme 1, Wang MicroTubes **1** were oxidized with IBX to aldehyde **2** and reduced back to the alcohol with LiBH$_4$. Using the modified dansylhydrazine method,[8] the reaction processes were monitored by measuring the increasing (oxidation) or decreasing (reduction) amount of aldehyde groups on the MicroTubes. Because the amount of IBX or LiBH$_4$ used in the reactions was over 10 times excess relative to the amount of functional groups on the MicroTubes, both reactions could be treated as a pseudo first-order reaction (see Experimental Section in the Supporting Information for detail).[11] The observed reaction rate constant (k_{obs}) can therefore be fitted with a semiempirical equation (eq 1)

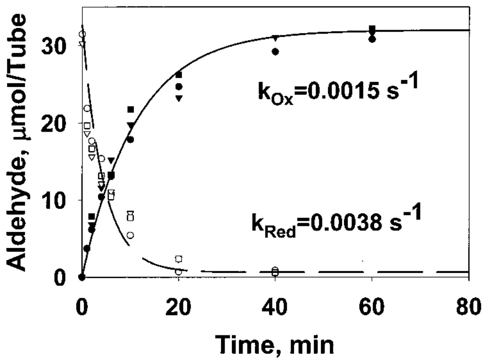
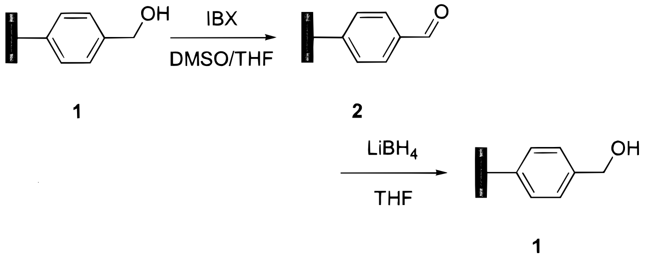


Figure 1. Three independent time courses of oxidation (solid) and reduction (open) on Wang MicroTubes. Experimental conditions: 50 mL of 0.1 M IBX in 1:4 DMSO/THF at 25 °C for oxidation reaction; 50 mM LiBH$_4$ in THF for reduction reaction. Lines are the theoretical fitting with an observed rate constant of 1.5 × 10^{-3} s^{-1} for oxidation reaction (solid line) or the fitting with an observed rate constant of 3.8 × 10^{-3} s^{-1} for reduction reaction (dashed line), respectively.

Scheme 1



$$y = a_0(1 - e^{-k_{obs}t}) \qquad (1)$$

where a_0 is the reaction conversion percentage when the reaction is completed and y is the measured reaction product percentage at each time point. Then, the observed reaction half time ($t_{1/2}$) and the observed 99% complete time (t_{99}) could be calculated with eq 2 and eq 3, respectively,

$$t_{1/2} = 0.69/k_{obs} \qquad (2)$$

$$t_{99} = 6.7t_{1/2} \qquad (3)$$

Figure 1 depicts the reaction time courses of the oxidation of “Wang” MicroTubes with 100 mM IBX in DMSO/THF (1:4) at 25 °C. An observed rate constant of 1.5 × 10^{-3} s^{-1} was fitted with eq 1. The observed half time ($t_{1/2}$) of 460 s was calculated based on three independent experiments (Figure 1 and Table 1). The average amount of the formed aldehyde is 32 μmol/MicroTube (Figure 1), and conversion of 98% was calculated (Table 1) based on the initial substitution level of 33 μmol/MicroTube.[12] Using Wang resin as a control, the same reaction conversion percentage was obtained under identical reaction conditions.[13]

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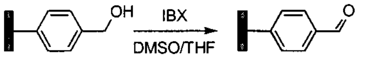
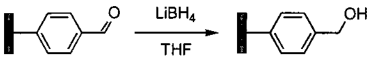
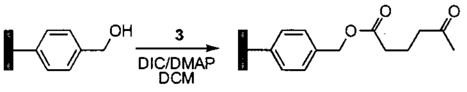
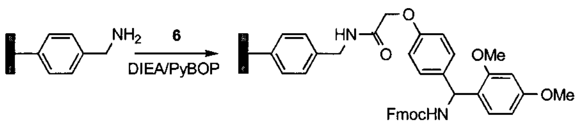
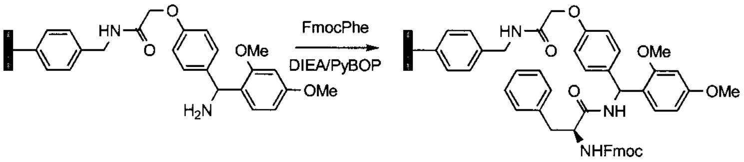
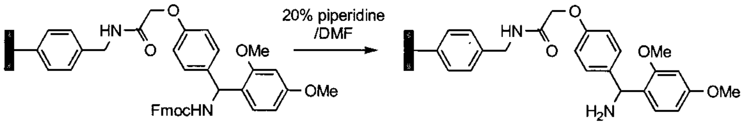
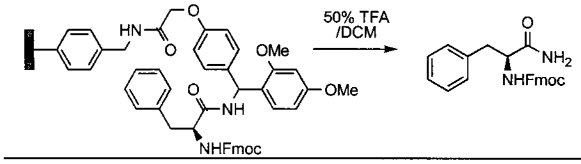
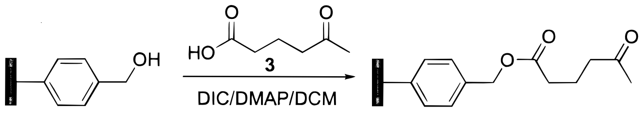
Table 1. Kinetics of Organic Reactions on Grafted MicroTubes

Reactions	k_{obs} (1/s)*	$t_{1/2}$ (min)	t_{99} (min)	Conversion (%)
	1.5×10^{-3}	7.7	52	98
	3.8×10^{-3}	3.0	20	98
	1.1×10^{-3}	10.5	70	98
	1.0×10^{-4}	115.5	770	93
	2.5×10^{-4}	46.2	310	90
	1.6×10^{-3}	7.2	48	93
	1.2×10^{-4}	96.2	642	97

*Using the concentration/conditions described in the Experimental Section.

Scheme 2



1 4

Figure 1 also depicts the decrease of aldehyde groups on the MicroTube when the aldehyde MicroTubes **2** were reduced with 50 mM $LiBH_4$ in THF. An observed rate constant of 3.8×10^{-3} s^{-1} was fitted with eq 1 based on three independent experiments (Figure 1). In addition, FTIR spectra confirmed that the aldehyde groups on the MicroTubes were completely reduced to hydroxyl groups.[14]

2. Ester Formation. Scheme 2 shows the coupling of Wang MicroTubes with 4-acetylbutyric acid **3** in DIC/DMAP/DCM, forming MicroTube-bound ester **4**. The reaction progress was monitored using the modified dansylhydrazine method by measuring the amount of bound ketone groups.[8]

As shown in Figure 2, an observed rate constant of 1.1×10^{-3} s^{-1} and an average amount of 32 μmol/MicroTube were fitted with eq 1 (Figure 2 and Table 1). Again, based on the initial loading of the Wang MicroTubes,[12] the reaction conversion percentage was 98% (Table 1).

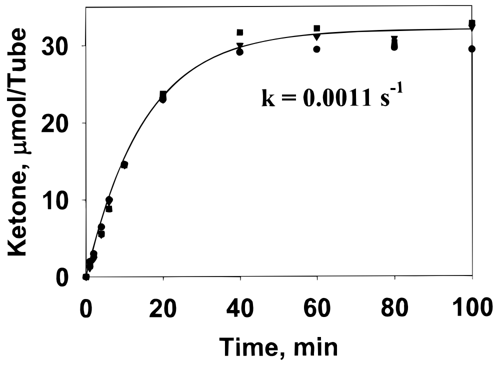
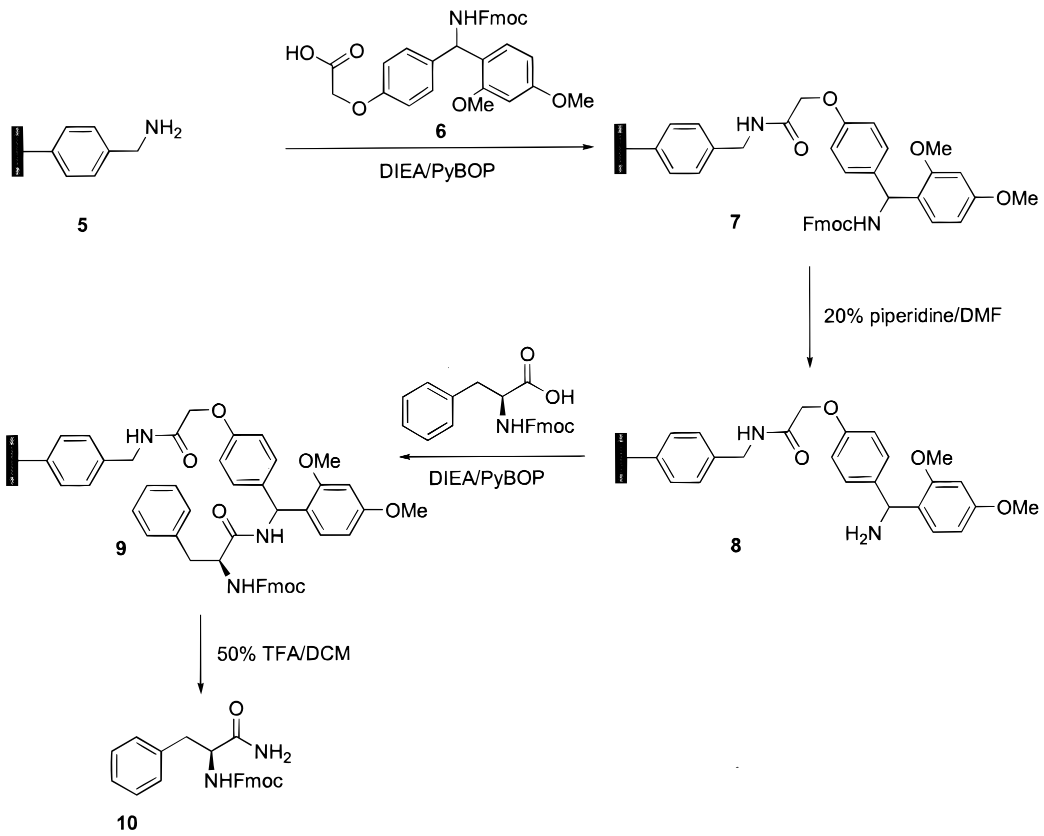


Figure 2. Three independent time courses of ester formation on Wang MicroTubes. Experimental conditions: 50 mL of 0.1 M 4-acetylbutyric acid/0.1 M DIC/0.1 M DMAP in DCM at 25 °C. Line is the theoretical fitting with an observed rate constant of 1.1×10^{-3} s^{-1}.

II. Organic Reactions on Aminomethylated (AM) MicroTubes. 1. Amide Formation and Fmoc Deprotection. Scheme 3 shows the attachment of Knorr linker **6** on aminomethylated MicroTubes **5**, Fmoc deprotection, and coupling with N-Fmoc-Phe-OH using PyBOP/DIEA/DCM.[15]

Scheme 3



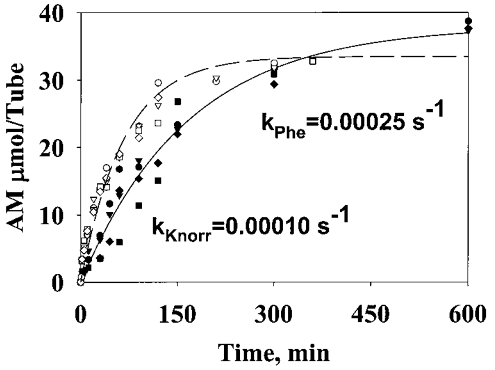


Figure 3. Four independent time courses of attachment of Knorr linker or coupling of Fmoc-Phe-OH on aminomethylated MicroTubes with 50 mL of 0.2 M Knorr/0.4 M DIEA/0.2 M PyBOP (solid) or 0.2 M Fmoc-Phe-OH/0.4 M DIEA/0.2 M PyBOP (open) in DCM at 25 °C. Lines are the theoretical fitting with an observed rate constant of 1.0×10^{-4} s^{-1} for the attachment of Knorr linker (solid line), or the fitting with an observed rate constant of 2.5×10^{-4} s^{-1} for coupling of Fmoc-Phe-OH (dashed line), respectively.

Both reactions were monitored by measuring the amount of bound Fmoc groups on the MicroTubes.[16]

Figure 3 shows the time courses of four independent experiments of Knorr linker attachment on AM MicroTubes using PyBOP/DIEA/DCM. An observed rate constant of 1.0×10^{-4} s^{-1} was fitted with eq 1. An observed half time ($t_{1/2}$) of 116 min and an observed 99% complete time of 770 min were calculated with eq 2 and eq 3, respectively (Figure 3 and Table 1). Although the amide formation on MicroTube is slow, the reactions still complete in a practical length of time with an average conversion of 93%.

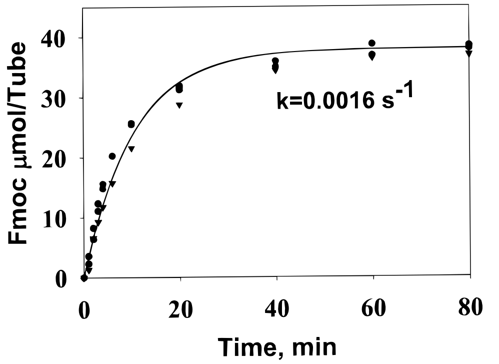


Figure 4. Three independent time courses of Fmoc deprotection on Knorr MicroTubes treated with 20% piperidine/DMF at 25 °C. Line is the theoretical fitting with an observed rate constant of 1.6×10^{-3} s^{-1}.

The Fmoc groups were then deprotected using 20% piperidine/DMF, and the process was monitored spectrophotometrically at 301 nm (extension coefficient: 7800 M^{-1} cm^{-1}).[16] As shown in Figure 4, the observed rate constant is 1.6×10^{-3} s^{-1}, and the observed 99% complete time is 48 min (Figure 4 and Table 1).

After Fmoc deprotection, aminomethyl MicroTubes **8** were further coupled with N-Fmoc-Phe-OH using PyBOP/DIEA/DCM, affording amide **9**. The observed rate constant (2.5×10^{-4} s^{-1}) is 2.5 times faster than that of the reaction for Knorr linker attachment on AM MicroTubes (Figure 3 and Table 1).

2. Fmoc-Phe-NH_2 Cleavage. MicroTubes **9** were cleaved with 50% TFA/DCM (Scheme 3). The amount of the cleaved product **10**, N-Fmoc-Phe-NH_2, was measured using Fmoc

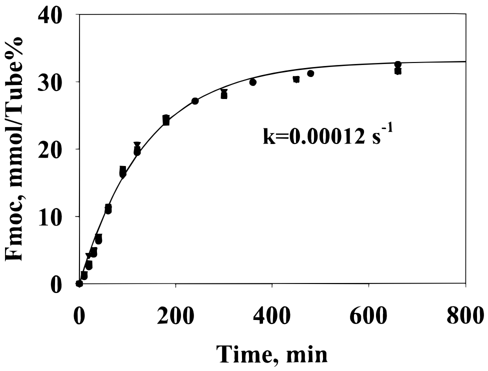


Figure 5. Time courses of cleavage by 50% TFA/DCM at 25 °C. Line is the theoretical fitting with observed rate constants of 1.2×10^{-4} s^{-1}.

analysis.[16] The structure of the cleavage product was confirmed by NMR and MS.[17] The purity of the final compound was over 90% as measured by NMR.

An observed cleavage rate of 1.2×10^{-4} s^{-1}, and an average loading of 33 μmol/MicroTube of Fmoc-Phe-NH_2 were fitted with eq 1 (Figure 5 and Table 1). Again, the cleavage reaction is slow but still can complete in a reasonable length of time with an average conversion of 97% (Figure 5 and Table 1). The overall yield (four steps) is over 80% based on the initial loading of the AM MicroTube (41 μmol/Tube as measured by Fmoc-Cl method, see Experimental Section in Supporting Information).

In summary, six common organic reactions have been evaluated on MicroTube solid supports. All reactions perform satisfactorily with over 90% conversion percentage and complete in useful lengths of time.

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Supporting Information Available. Experimental Section including details of the synthesis on Wang and AM MicroTubes, measurement procedures, and kinetic analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Abbreviations. IBX: 1-hydroxy-1,2-benziodoxol-3(1*H*)-one-1-oxide; DIC: 1,3-diisopropylcarbodiimide; DMAP: 4-(dimethylamino)-pyridine; DMSO: dimethyl sulfoxide; THF: tetrahydrofuran; DMF: *N,N*-dimethylformamide; DCM: dichloromethane; DIEA: *N,N*-diisopropylethylamine; PyBOP: benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; Knorr: 4-[(*R,S*)-α-[1-(9*H*-fluoren-9-yl)-methoxy-formamido]-2,4-dimethoxybenzyl-phenoxyacetic acid; TFA: trifluroacetic acid; HOAc: acetic acid; $LiBH_4$: lithium borohydride.

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(10) Li, W.; Xiao, X.; Czarnik, A. W. Kinetic Comparison of Amide Formation on Various Cross-Linked Polystyrene Resins. *J. Comb. Chem.* **1999**, *2*, 151−153.

(11) All reactions in this paper were treated as pseudo first-order reactions.

(12) Loading of 32.6 μmol/Tube was calculated from chloride measurement reported in ref 5.

(13) This method can be applied as a routine loading measurement for Wang type of MicroTubes.

(14) IR spectra indicated that a carbonyl peak of the newly formed aldehyde group appeared at 1693 cm^{-1} while the broad hydroxyl peak of WangTube at 3400 cm^{-1} disappeared when WangTubes were oxidized by IBX. This carbonyl peak disappeared with the addition of $LiBH_4$.

(15) While MicroTubes showed excellent performance in DCM and THF for many reactions, slower kinetics was observed for amide formation in DMF.

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(17) Compound **10**: $C_{24}H_{22}N_2O_3$ (MW: 386). MS: $[MH^+] = 387$. ^1H NMR (DMSO-d_6): δ 7.88 (d, 2H), 7.64 (t, 2H), 7.46 (d, 2H), 7.41 (t, 2H), 7.27 (m), 4.40 (d, 2H), 4.18 (m), 3.02 (d, 2H), 2.79 (t, 1H), 1.24 (t, 1H).

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